Monday, October 3, 2016 (Christine Tang)

Objectives: Continue invasin gibson assembly from September 28, 2016
Finish gibson assembly with parts 1+2, 3+4 and pFd007.Tet template
Do another Gibson with individual parts of template at the same time

1. For objective 1 and 2, referred to procedure on "Creating Constructs for Mammalian Cells" and the entry for September 28, 2016.*

Part	Concentration	Length
Invasin 1+2	10.8 ng/µL	1,764 bp
Invasin 3+4	16.8 ng/µL	1,566 bp
pFd007.Tet	77.3 ng/µL	2846 bp

Note:* did not proceed with gibson assembly of these parts because concentrations are too low.

2. For objective 3, see step #1. Used 6 µL of DNA and 18 µL of isothermal ligase buffer.

Part	Concentration	Length
Invasin 1	333.9 ng/µL	945 bp
Invasin 2	245.4 ng/µL	819 bp
Invasin 3	189.2 ng/µL	861 bp
Invasin 4	227.2 ng/µL	705 bp
pFd007.Tet	77.3 ng/µL	2846 bp

3. Transformed the completed pFd007.Tet and invasin parts 1-4 from step #2 in XLIEC (electro-competent) *E. coli*. Referred to the Electroporation protocol.
4. Plated 100 µL, 1:100 and 1:1000 dilutions on lB/CM (chloramphenicol) plates. Incubated for 18 hours at 37 degrees Celsius.

Conclusion: Will colony PCR later (see October 9, 2016 entry).

Tuesday, October 4, 2016 (Margaret Lie and Albert Truong)

Objectives: Set up PCR of fdhf backbone, iRFP670, and iRFP713
Finish Golden Gate DNA Assembly
Transform and plate cells.

1. Set up PCRs
    a.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 80(20x)	2.5 µL	10 µM
iGEM 78 (20x)	2.5 µL	10 µM
Fdhf backbone #5	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
60.4	0:30	25-35X
72	1:06	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

    b.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 81(20x)	2.5 µL	10 µM
iGEM 83 (20x)	2.5 µL	10 µM
Fdhf backbone #5	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
58.4	0:30	25-35X
72	0:30	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

c.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 77 (20x)	2.5 µL	10 µM
iGEM 79 (20x)	2.5 µL	10 µM
pBAD/HisB/iFP713	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (^{o}C)	Time	Cycle
98	0:30	
98	0:10	
61.8	0:30	25-35X
72	0:30	
72	10:00	
4	∞	

2. The primer design for b. Is not ideal because the difference is 4.4 degrees Celsius.
3. Ran 0.8% agarose gel at 110 V for 25 minutes.
    a. Saw multiple bands so will gel purify.
4. Incubated 1h at 37^{O}C with Dpn1.
5. Ran 0.8% agarose gel at 110 V for 25 minutes.
    a. Accidently cut through 670 band.
    b. All bands present
6. Used Zymo Gel Purification Kit (Irvine, CA). Accidently, added DNA Binding Buffer to 670 but then properly used ADB.
7. Used Nanodrop to find concentrations.
8. Set up Golden Gate DNA Assembly
9. Golden Gate DNA Synthesis
    a. **Reaction Setup**

1.36 µl	Largest DNA piece (~100 ng) from PCR product fdhf2
X µl	0.36 µl Cyan 0.92 µl 670 0.38 µl Anacy 0.94 µl 713
1.5 µl	10x NEB T4 Buffer
1.5 µl	10x BSA*

0.5 µl	BsaI
0.5 µl	NEB T4 Ligase, 400.000 cohesive end units/ µl
QS 15 µl	dH2O

b. *Without the addition of BSA *Bsa*I is only 10% active at 37°C

c. **Thermo Cycling**

Temp. (°C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

Summary: I was disappointed with the nanodrop because the max concentration was not clustered at around 260 nm. Hopefully, it works. I will Zymo Clean and Concentrate tomorrow and transform for plating on LB/Cm(34). Also, I wish that my primers would come tomorrow.

Wednesday, October 5, 2016 (Margaret Lie and Kevin Chen)

Objectives: Transformation of Golden Gate DNA Assembly products

1. Purified DNA with Zymo Clean and Concentrate Kit (Irvine, CA).
2. Transformed following into 40 µL *E. coli* XL1 by electroporation.
    a. pSB1C3/fdhf/iRFP670
    b. pSB1C3/fdhf/iRFP713
    c. pSB1C3/fdhf/anacy_2551g3
    d. pSB1C3/fdhf/cyan7822_4053g2
3. Incubated 1 h at 37 degrees Celsius and 250 rpm in 1 mL SOC.
    a. Time: 1:45 P.M. to 2:45 P.M.
    b. Thawed 4 LB/Cm(34) plates in incubator (2:25 P.M.)
    c. Labelled plates:
        i. pSB1C3/fdhf/iRFP670 in XL1 EC 100 microliters KC 5 Oct 2016
        ii. pSB1C3/fdhf/iRFP713 in XL1 EC 100 microliters KC 5 Oct 2016
        iii. pSB1C3/fdhf/anacy_2551g3 in XL1 EC 100 microliters KC 5 Oct 2016
        iv. pSB1C3/fdhf/cyan7822_4053g2 in XL1 EC 100 microliters KC 5 Oct 2016
4. Plated 100 microliters. Incubated 16-18 h at 37 degrees Celsius (3:00 P.M.)
5. Re-suspended primers iGEM 100-111. Stored in Primers Box 3 at -20 degrees Celsius.
6. Set up PCR reactions.

a.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 104(20x)	2.5 µL	10 µM
iGEM 106 (20x)	2.5 µL	10 µM
Anacy gBlock	Variable (~0.5uL)	
Phusion DNA Polymerase	1 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
61.9	0:30	25-35X
72	0:18	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

b.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM108 (20x)	2.5 µL	10 µM
iGEM109 (20x)	2.5 µL	10 µM
pBAD/HisB/iFP713	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
59.1	0:30	25-35X
72	0:19	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

c.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM105 (20x)	2.5 µL	10 µM
iGEM107 (20x)	2.5 µL	10 µM
pBAD/HisB/iFP713	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
63.3	0:30	25-35X
72	2:04	
72	10:00	
4	∞	

*I messed up designing these primers iGEM 105 has a ™ of 69.4

*Round down Tm for lowest annealing temperature primer

Thursday, October 6, 2016 (Margaret Lie, Jonathan Pan, Kevin Chen)

1. Ran 0.8% gel at 110 V for 20 minutes.
    a. 670 approx. 4.109 kb
    b. Anacy approx. 591 bp
    c. Cyan approx. 603 bp
2. Incubated 670 with 1 microliter Dpn1 at 37 degrees Celsius for 1 h.
3. Ran 0.8% gel at 110 V for 20 minutes. Used Zymo Gel Purification Kit.
4. Set up PCR:
    a.

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM110 (20x)	2.5 µL	10 µM
iGEM111 (20x)	2.5 µL	10 µM
pBAD/HisB/iFP713	Variable (~1uL)	
Phusion DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
58.4	0:30	25-35X
72	2:32	
72	10:00	
4	∞	

5. Set up colony PCR for Fdhf/670, 713, anacy, cyan
    a. Made Master Mix (Vent/TAQ) (supplies 20 colony reactions):

Material	Volume	Concentration
dH20	134µL	-
ThermoPol Buffer	20 µL	-
dNTPs (50x)	4 µL	10 mM
VF (20x)	10 µL	10 µM
VR (20x)	10 µL	10 µM
Vent DNA Polymerase	2 µL	100x stock

Thermocycling for 670, 713:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
58.6	0:30	35X
68	0:42	
68	5:00	
4	∞	

Thermocycling for anacy, cyan:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
58.6	0:30	35X
68	0:30	
68	5:00	
4	∞	

b. Scraped 5 colonies from each plate and streaked on new plate with toothpick.
c. Suspended colonies in 20µL of dH2O using toothpick
d. Transfered toothpick to 3mL LB + Cm
e. Added 1µL of cell suspension to 9µL master mix

f. Incubated LB+Cm cultures overnight for 16 hours at 37 degrees Celsius.
g. Incubated plate with all 20 streaks overnight for 16 hours at 37 degrees Celsius.

Friday, October 7, 2016 (Margaret Lie)

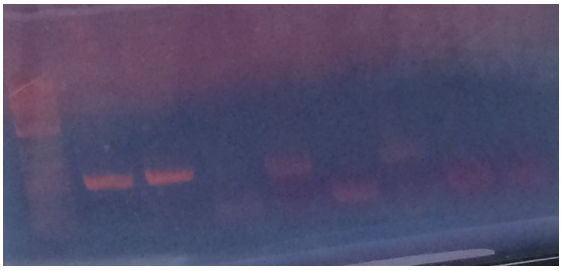
6. Nanodropped to find concentration.
7. Set up Golden Gate.
    a. Tube 1:
        i. 0.54 microliter 100 ng pBAD/HisB/iRFP670 PCR (w/ 110 & 111 primers)
        ii. 1 microliter Bbs1
        iii. 1.5 microliter T4 ligase buffer
        iv. 1 microliter T4 ligase
        v. 10.96 microliters dH_2O
    b. Tube 2:
        i. 0.92 microliter 100 ng pBAD/HisB PCR (w/ 105 & 107 primers)
        ii. 0.35 microliter 44 ng Cyan PCR
        iii. 1 microliter Bbs1
        iv. 1.5 microliter T4 ligase buffer
        v. 1 microliter T4 ligase
        vi. 10.23 microliters dH_2O
    c. Tube 3:
        i. 0.92 microliter 100 ng pBAD/HisB PCR (w/ 105 & 107 primers)
        ii. 0.32 microliter 43.1 ng Anacy PCR
        iii. 1 microliter Bbs1
        iv. 1.5 microliter T4 ligase buffer
        v. 1 microliter T4 ligase
        vi. 10.26 microliters dH_2O
    d. Thermocycle:
        i. **Thermo Cycling**

Temp. (^oC)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

8. Used Zymo Clean and Concentrator
9. Transformed into 40 microliters XL1EC by electroporation.

10. Incubated 1 h at 37 degrees Celsius and 250 rpm.
11. Plated 100 microliters on LB/Amp(100). Incubated overnight at 37 degrees Celsius.

Friday, October 7, 2016 (Kevin Chen)

1. Placed plate with 20 streaks in 4 degree fridge.
2. Miniprepped overnight cultures.
3. Ran 0.8% agarose gel at 110 V for 15 minutes of the previous night's colony PCRs.
4.  

5 670s, 5 713's, 1 anacy 4 anacies, 4 cyans (1 cyan misplaced)

Sunday, October 9, 2016 (Kevin Chen, Christine Tang)

Objective: PCR and grow cultures of pBAD/HisB-iRFPs. **(Kevin Chen)**

1. Set up colony PCR for iRFP anacy and cyan:
    a. Made 2 Master Mixes (Vent/TAQ) (each supplies 5 colony reactions):

Material	Volume	Concentration
dH20	33.5 µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM104(20x)	2.5 µL	10 µM
iGEM106(20x)	2.5 µL	10 µM
Vent DNA Polymerase	0.5 µL	100x stock

Material	Volume	Concentration
dH20	33.5 µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM108(20x)	2.5 µL	10 µM
iGEM109(20x)	2.5 µL	10 µM
Vent DNA Polymerase	0.5 µL	100x stock

b. Scraped 4 colonies from each plate (including pBAD/HisB-iRFP670) and streaked on new plate with toothpick.

c. Suspended colonies of Anacy and Cyan in 20µL of dH2O using toothpick

d. Transfered toothpick to 3mL LB + Amp

e. Added 1µL of cell suspension to 9µL master mix

Thermal Cycling:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
59.1 for cyan, 61.9 for anacy	0:30	35X
68	0:18	
68	5:00	
4	∞	

f. Incubated the LB+amp cultures overnight for 16 hours at 37 degrees Celsius.

g. Incubated Plate with all 12 streaks overnight for 16 hours at 37 degrees Celsius.

Objective: Colony PCR invasin (see entry on October 3, 2016) **(Christine Tang)**

1. Followed Colony PCR protocol. Grew 3 cultures from 100 µL (undiluted) plate from October 3, 2016.
    a. Forward primer: Gibson 1
    b. Reverse primer: Gibson 7
2. Made 3 bacteria cultures (CM) and 3 PCR samples. Ran 0.8% agarose gel at 110 V for PCR samples.
3. Incubated bacteria cultures for 18 hours at 37 degrees Celsius.

Conclusion: Will proceed to miniprep tomorrow and send for sequencing.

Monday, October 10, 2016 (Kevin Chen, Christine Tang)

1. Took plates out of incubator.
2. Miniprepped overnight cultures.
3. Ran 0.8% agarose gel for yesterday's cyan and anacy colony PCRs at 110 V for 30 minutes. Anacy bands were far too small compared to the ladder (below 100 bp), and no cyan bands appeared. Still decided to sequence cyan bands anyway in case of PCR error.
4. Nanodropped minipreps for Fdhf and pBAD plasmids from Friday and today.
    a. Fdhf 670 #2: 135.5 ng/uL
    b. Fdhf 670 #3: 102.8 ng/uL

c. Fdhf 713 #2: 139.8 ng/uL
d. Fdhf 713 #3: 216.2 ng/uL
e. Fdhf anacy 1: 194.9 ng/uL
f. Fdhf anacy 2: 257.3 ng/uL
g. Fdhf anacy 3: 77.5 ng/uL
h. Fdhf cyan 2: 236.9 ng/uL
i. pBAD 670 1: 114.9 ng/uL
j. pBAD 670 2: 204.0 ng/uL
k. pBAD 670 3: 200.2 ng/uL
l. pBAD 670 4: 161.0 ng/uL
m. pBAD cyan 2: 495.8 ng/uL
n. pBAD cyan 3: 173.0 ng/uL
o. pBAD cyan 4 136.5 ng/uL

5. Sent pBAD/iRFP670,cyans Fdhf/iRFP670,anacy,cyans and invasin for Genewiz sequencing.

Will redo pBAD anacy and cyan colony PCRs tomorrow. (would do today but tomorrow's morning availability between all wet lab members is questionable)

Objective: Miniprep invasin cultures and send for sequencing **(Christine Tang, Kevin Chen)**

1. Took bacteria cultures out of incubator. Miniprepped all 3 cultures.
2. Sent to Genewiz for sequencing with 4 different primers: gibson 1, gibson 3, gibson 10 and iGEM 33. Used 500 ng of purified DNA sample and 2 µL of a sequencing primer. Sent a total of 8 invasin samples for sequencing.

Conclusion: Will get sequencing results tomorrow.

Tuesday, October 11, 2016 (Christine Tang)

Objective: Checked sequencing for invasin samples from October 10, 2016.

Conclusion: Only 1 out of the 8 samples sent for sequencing passed quality control. Will redo in the future.

Wednesday, October 12, 2016 (Kevin Chen and Margaret Lie)

1. Checked sequencing
    a. Fdhf 670 #2 ok
    b. Fdhf 713 #3

c. Fdhf anacy #1 ok
a. Fdhf cyan #2 ok
b. pBAD 670 #3 ok
c. pBAD cyan #2 ok

2. Grew overnight cultures for pBAD anacy from master plate from original pcr, grew overnight cultures of ppcb6 from previous plate (6:30 PM)
3. Transformed into rosetta Fdhf 670 and Fdhf 713 and into psr34 pBAD 670. Plated the Fdhf plasmids onto a Cm plate, letting incubate overnight. Plated the pBAD 670 onto a amp/strep plate, letting incubate overnight. (7:40 PM)
4. Transformed following into 40 µL *E. coli* E. Rosetta by electroporation.
   a. pfdhf/anacy + pPCB6
   b. pfdhf/cyan + pPCB6
   c. pBAD/cyan + pPCB6
   d. pBAD/713 + pSR34Bvd
5. Incubated 1 h at 37 degrees C and 250 rpm in 1 mL SOC.
6. Plated 100 microliters of pfdhf/anacy + pPCB6 and pfdhf/cyan + pPCB6 on LB/Strep(100)/Cm(34). Plated 100 microliters of pBAD/cyan + pPCB6 and pBAD/713 + pSR34Bvd on LB/Strep(50)/Amp(50).

Summary: will miniprep/sequence pBAD anacy tomorrow, and miniprep pPBC6 tomorrow. Transformations will be taken out tomorrow after 16-18 hours - will colony PCR and figure out rest later with Margaret.

Thursday, October 13, 2016 (Margaret Lie)

1. Miniprepped pBAD/anacy.
   a. Unable to miniprep pPCB6 because cultures did not grow.
   b. All plates Kevin plated had colonies. Kept the 1:1000 dilutions.
   c. pfdhf/670, pfdhf/713, pBAD/670 ready for assay.
   d. Note to Kevin: Please spread the culture better on plates. Spread the bacteria more evenly so it does not clump.
   e. Sent for sequencing after nanodrop to find concentration.
2. Prepared LB agar.
3. Plated all of following on either LB/Strep(50)/Cm(17) or LB/Strep(50)/Amp(50)
   a. pfdhf/anacy + pPCB6
   b. pfdhf/cyan + pPCB6
   c. pBAD/cyan + pPCB6
4. Transformed pPCB6 into *E. coli* E. Rosetta. Plated on LB/Strep(50).
5. Grew cultures of pPCB6 in 3 mL LB/ Strep(50).

6. Grew 3 mL cultures of pfdhf/670 and pfdhf/713 in 3 mL LB/Cm(34). Grew 3 mL cultures of pBAD/670 and pBAD/713 in 3 mL LB/Amp(50)/Strep(50).
    a. **Start this after 4:30 P.M.**
    b. **Was 6pm**

Friday, October 14, 2016 (Kevin Chen, Margaret Lie, Jonathan Pan, Christine Tang)

Objective:

1. For fdhf/670 and fdhf/713, 1:100 dilution in glass vials with stoppers.
    a. 5 mL LB/Cm(34)
    b. 16 microliters BV (yes, pg 20)
    c. 50 microliters culture
2. 3 vials x hypoxic and 3 vials x with oxygen
3. Cover vials with aluminum foil.
4. Grow for 24 hours at 37 C in dark incubator.
5. 11:30am on average (ranged from 11:15 for 1st 2 to 11:40 for next 2 to 11:58 for next 2)

Objective:

1. For pBAD/670+pSR34Bvd and pBAD/713+pSR34Bvd, 1:100 dilution in 50 mL flask
    a. 25 mL LB/Amp(50)/Strep(50)
    b. 250 microliters culture
2. Write what time you put in incubator: 10:45 A.M.
3. Induced at with 2 mM arabinose at 1:15 P.M.

Objective: to redo colony PCR for invasin (see entry on October 11, 2016) **(Christine Tang)**

1. Picked 20 colonies from the invasin bacteria plate from the 100 µL (undiluted) invasin plate and did colony PCR. Followed the Colony PCR protocol.
2. Made 40 PCR samples with the 20 colonies using two different sets of primers to amplify either invasin parts 1 and 2 (primer pair: gibson 1 and gibson 6) or parts 3 and 4 (primer pair: gibson 5 and gibson 7).
3. **Reaction Setup and Thermocycling**:

    For Master Mix (this is for 20 samples for 1 primer pair):

Material	Volume
5x Phusion Buffer	20 µL
DTNP	4 µL
Forward primer (either gibson 1 or gibson 5)	10 µL

Reverse primer (either gibson 6 or gibson 7)	10 µL
Phusion Polymerase	2 µL
Nuclease-free water	134 µL

Temp. (°C)	Time	Cycle
98	5:00	
98	0:30	
98	0:10	
45-72*	0:30	25-35X
72	0:30/kb	
72	10:00	
4	∞	

4. Incubated the 20 cultures at 37 degrees Celsius.

Conclusion: Will run a gel of the PCR products. Will miniprep cultures and send for sequencing.

Saturday, October 15, 2016 (Christine Tang, Rohan Bhardwaj, Margaret Lie)

Objective: To continue making fdhf/670, mNarK/670 and pSoxS/670 Biobrick Compatible.

1. Nanodrop for concentration. **(Christine Tang)**
    a. pfdhf/670 #2 = 36.8 ng/µl
    b. mNarK/670 = 45.4 ng/µl
    c. pSoxS/670 = 15.2 ng/µl
2. Set up Golden Gate DNA Assembly. **(Christine Tang)**
    a. **Reaction Setup: You need 3 tubes.**

µl	DNA piece (~100 ng) from PCR product
1.5 µl	10x NEB T4 Buffer
1.0 µl	Bbs1
1.0 µl	NEB T4 Ligase, 400.000 cohesive end units/ µl
QS 15 µl	dH2O

    a. *Without the addition of BSA *Bsa*I is only 10% active at 37°C

b. **Thermo Cycling**

Temp. (°C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

Objective: To run invasin colony PCR products on gel to check sizes, miniprep and send for sequencing **(Christine Tang)**

1. Ran all 40 PCR samples (from October 14, 2016) on a 0.8% agarose gel at 110 V.

Conclusion: None of the PCR samples showed a band at 1.7 or 1.5 kb (depending on which set of primers were used). Did not miniprep the 20 bacteria cultures.

1. Assay fdhf
    a. 670, 713 = Thursday - Saturday
    b. Anacy, cyan = Friday - Sunday at best
        i. Transform pPCB
        ii. Friday cultures
        iii. Saturday miniprep
        iv. Sunday transform
        v. Monday grow cultures
        vi. Tuesday induce
        vii. Wednesday data
2. Assay pBAD
    a. 670, 713 = Thursday - Saturday
    b. Cyan = Friday - Sunday at best
    c. Anacy transform Friday, Cultures Saturday

Whoever's here in lab today: Start the gel for #3 or start the PCR for #4

3. Set up PCR of (to make biobrick compatible) Thursday/Friday- Monday/Tuesday
    a. pfdhf/670 #2 = 1:50 dilution
        i. T_m = 58.4 C

ii. Product size = 3137 bp

b. mNarK/670 = hypoxia
   i. T_m = 58.4 C
   ii. Product size = 3171 bp
c. pSoxS/670 = 1:100 dilution
   i. T_m = 58.4 C
   ii. Product size = 3859 bp

c.

dH20	32.0 QS 50µL	-
High GC Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 110(20x)	2.5 µL	10 µM
iGEM 111 (20x)	2.5 µL	10 µM
Template	Variable (1 uL)	
Phusion DNA Polymerase (100x)	1 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
58.4	0:30	25-35X
72	pfdhf/670 1:35 mNarK 1:36 pSoxS 1:56	
72	10:00	
4	∞	

d. Pour 0.8% agarose gel. Run 5 microliters of sample + 1 microliter Gel star loading dye and 10 microliters of ladder.
   i. 110 V for 20 minutes
   ii. Store the rest at 4 C.
e. If bands are the correct size, incubate the stored sample for 1 h at 37 C with 1 microliter Dpn1.
f. Zymo Clean and Concentrate DNA → Can stop here or can stop at the next zymo step.

Update at night: ended up gel extracting remaining 45 uL, did gel recovery kit but no one could find DpnI so we didn't DpnI it tonight. **(Kevin Chen)**

g. Nanodrop for concentration. **(Christine Tang)**
   i. pfdhf/670 #2 = 36.8 ng/µl
   ii. mNarK/670 = 45.4 ng/µl
   iii. pSoxS/670 = 15.2 ng/µl
h. Set up Golden Gate DNA Assembly. **(Christine Tang)**

i. **Reaction Setup: You need 3 tubes.**

µl	DNA piece (~100 ng) from PCR product
1.5 µl	10x NEB T4 Buffer
1.0 µl	Bbs1
1.0 µl	NEB T4 Ligase, 400.000 cohesive end units/ µl
QS 15 µl	dH2O

i. *Without the addition of BSA *Bsa*I is only 10% active at 37°C

j. **Thermo Cycling**

Temp. (°C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

I i. Zymo Clean and Concentrate

J. Transform into XL1 EC.

Monday, October 17th, 2016

1. PCR pBAD 670 #3 ok, pBAD 713
    a. 1:22 time, 58.7 annealing temp. Phusion. iGEM 102 and 103
2. Ran 0.8% agarose gel with 5 uL to test size, 110 V for 20 minutes.
3. If bands are the correct size, incubate the stored sample for 1 h at 37 C with 1 microliter Dpn1. STOPPED HERE BEFORE ADDING DPNI, NOT SURE WHAT TO DO EMAILED MARGARET
4. Zymo Clean and Concentrate DNA → Can stop here or can stop at the next zymo step.

Grew 1 3mL pPCB6 culture for future transforming of the following into 40 µL *E. coli* E. Rosetta by electroporation. Let grow for 18 hours (12:30AM, so 4:30PM-6:30PM ok)

Transformed Fdhf/670, soxR/S670, NarK670. Plated 1:1000 of each formation onto Lb/Cm plates. Incubated at 37C for 16-18 hours (3:15 AM, take out at 7:15-9:15PM).